DATA EVALUATION RECORD
§ 72-1(C) -- ACUTE LC_{50} TEST WITH A COLDWATER FISH

1. **CHEMICAL:** Esfenvalerate PC Code No.: 109303

2. **TEST MATERIAL:** DPX-YB656-82 (H-20247) Purity: 44.4 % (SS isomer)

   Wettable powder formulation of esfenvalerate which contains a mixture of four optical isomers (total isomer content is approximately 51.0 %).

3. **CITATION**

   Authors: Kevin N. Baer, Ph.D.
   Title: Flow-through, acute, 96-hour LC50 of DPX-YB656-82 to rainbow trout, Oncorhynchus mykiss.
   Study Completion Date: June 13, 1994
   Laboratory: E. I. du Pont de Nemours and Company
   Haskell Laboratory for Toxicology and Industrial Medicine
   Sponsor: E. I. du Pont de Nemours and Company
   Laboratory Report ID: 254-94
   MRID No.: 433583-11
   DP Barcode: D208377

4. **REVIEWED BY:** William Rabert, Biologist, EEB, EFED

   Signature: William S. Rabert **Date:** 12/21/94

5. **APPROVED BY:** Dan Rieder, Section Head 3, EEB, EFED

   Signature: Daniel Rieder **Date:** 12-28-94

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: Oncorhynchus mykiss
   Age or Size of Test Organism: juvenile rainbow trout 3.2 (range 2.7-3.7 cm)
   Definitive Test Duration: 96 hours
   Study Method: Flow-through
   Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

   Results Synopsis: Based on EPA criteria, DPX-YB656-82 was very highly toxic to juvenile rainbow trout, Oncorhynchus mykiss, in a flow-through, acute, 96-hour test. The 96-hour LC50, based on measured, total isomer concentrations is 0.070 ppb ai.

   LC_{50}: 0.070 ppb ai 95% C.I.: 0.057-0.087 ppb ai
   NOEL: < 0.0266 ppb ai Probit Slope: 7.0

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. Guideline Deviations

1. Temperature measurements made only once every 24 hours, but a water bath was used to modify temperature changes.

2. Calibration and daily check of diluter was not reported.

3. Survival of organisms in dilution water was not reported. Description of chemical characteristics of well water is insufficient to assess toxic interferences. Levels of detection of metals and priority pollutants are too high compared to recommended limits by Stephan (1975).

10. SUBMISSION PURPOSE: Support of new formulation of Asana.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is the rainbow trout (Oncorhynchus mykiss)	Rainbow trout Oncorhynchus mykiss
Mean Weight 0.5-5 g	Mean: 0.40 g Range: 0.21 - 0.66 g
Mean Standard Length Longest not > 2x shortest	Mean: 3.2 Range: 2.7 - 3.7 cm
Supplier	Aquatic Research Organisms Hampton, New Hampshire
All fish from same source?	Yes
All fish from the same year class?	Yes

B. **Source/Acclimation**

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	39 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	48 hours prior to test
Pretest Mortality < 3% mortality 48 hours prior to testing	0 % mortality prior to testing.

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Haskell Laboratory well
Does water support test animals without observable signs of stress?	Not reported
Water Temperature 12°C	13.1 - 14.1 °C
pH Prefer 7.2 to 7.6	7.1 - 7.4
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	9.7 mg/L at 96 hours (94 % saturation)
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	89 mg/L as $CaCo_3$

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	Glass aquaria 10 L. 20 x 20 x 25 cm 7.4 L./5 fish
Type of Dilution System Must provide reproducible supply of toxicant	Brung's proportional diluter (glass)
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	10 vol./24 hours; no validation of flow rates at start or daily
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	controls reported as 0.028 g/L and averaged 0.0027 g/L/day
Photoperiod 16 hours light, 8 hours dark	16:8 hours
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: acetone Maximum conc.: 0.15 ml/L. [slightly exceeds 0.1 ml/L for flow-through tests]

D. **Test Design**

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	No indication of range finding test.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	0.054, 0.091, 0.15, 0.25, 0.42 and 0.70 ug ai/L (total formulation).
Number of Test Organisms Minimum 10/level, may be divided among containers	2 replicates of 5 fish each.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and Ph Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Held in a water bath; measured every 24 hours in all reps. measured every 24 hours in all reps.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Measured using GC.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0 %
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppb)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	--	10	0	0	0	0
Solvent Control	--	10	0	0	0	0
0.054	0.0266	10	0	0	0	0
0.091	0.0470	10	0	0	1	1
0.15	0.0740	10	0	2	6	6
0.25	0.112	10	0	7	8	9
0.42	0.225	10	2	10	10	10
0.70	0.266	10	10	10	10	10

Other Significant Results:

Sublethal effects occurred at all test concentrations beginning at 24 hours throughout test. Sublethal effects include: erratic swimming, gasping for air, and lying on bottom.

B. Statistical Results

Method: Probit Analysis

96-hr LC_{50}: 0.070 ppb ai 95% C.I.: 0.057-0.087 ppb ai

Probit Slope: 7.0_ NOEC: < 0.054 ppb ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	0.068 (0.047-0.112) ppb ai
Moving Average Angle LC_{50} (95% C.I.)	0.074 (0.058-0.092) ppb ai
Probit LC_{50} (95% C.I.)	0.070 (0.057-0.087) ppb ai
Probit Slope	6.95
NOEC	< 0.0266 ppb ai

14. REVIEWER'S COMMENTS:

The test results indicate that Asana SP Insecticide formulation is very highly toxic to a coldwater fish species. Test results support reported author's conclusions. Deviations from test protocols would not appear to have affected the test results.

The concentrations of the calibration curve did not span the range of test concentrations. The calibration curve constructed in order to determination of test concentrations ranged from 10.9 to 109 ug/L, while the measured test concentrations ranged from 0.0266 to 0.266 ug/L. All test concentrations fell 50 to 500 fold below the range of levels used for the calibration curve. All test concentrations are well within the levels of quantification (LOQ, 0.003 ug/L) and detection (LOD, 0.001 ug/L) for esfenvalerate.

DuPont has extrapolated test concentrations from calibration curves for all three acute toxicity tests that they submitted on Asana SP formulation. Paul Mastradone, EFGWB, indicated that while the test concentration measurements may be accurate, if the calibration curve is linear. Ideally, the calibration curve should span the range of test concentrations. EEB suggests that Registration Division contact and request that the registrant address this issue in future tests.

William Rabert Asana Rainbow Trout 96-hour LC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.266	10	10	100	9.765625E-02
.225	10	10	100	9.765625E-02
.112	10	9	90	1.074219
.074	10	6	60.00001	37.69531
.047	10	1	10	1.074219
.0266	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .047 AND .112 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 6.812496E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	6.987813E-02	7.465666E-02	
5.846004E-02		9.241741E-02	

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2861262	1	.9973729

SLOPE = 6.951908

95 PERCENT CONFIDENCE LIMITS = 3.233279 AND 10.67054

LC50 = 7.041832E-02

95 PERCENT CONFIDENCE LIMITS = 5.676504E-02 AND 8.670932E-02

LC10 = 4.623832E-02

95 PERCENT CONFIDENCE LIMITS = 2.647044E-02 AND 5.722377E-02

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